ABNORMAL CYTOLOGY OF EPITHELIAL CELLS IN PEMPHIGUS VULGARIS: A DIAGNOSTIC AID*

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There has been a pressing need for a reliable diagnostic test for pemphigus vulgaris. Although some cases have unmistakable clinical features, in many patients it is difficult to distinguish between pemphigus and other more benign diseases which exhibit vesicles and bullae of the skin and mucous membranes.

Although the etiology is unknown, various serologic and chemical diagnostic procedures have been advocated in the past. These have not been reliable and now are justly in ill repute. Attention repeatedly has been focused on the bullae as the most apparent feature of this disease. The histology of these lesions has been studied carefully for evidence of any characteristic and diagnostic changes. Many observers concluded that under the microscope the bullae of pemphigus were indistinguishable from other bullae. A few pathologists insisted that the bullae were predominantly intraepidermal in pemphigus (6) but the study of serial sections by Percival and Hannay demonstrated that even in pemphigus, part of the floor of the bulla may be free of epithelial cells (8).

Civatte, in recent years, has described the detailed cytologic changes of pemphigus epithelial cells undergoing acantholysis and degenerative changes (3, 4). He also demonstrated in histologic sections that the bullae appear to originate in the prickle layers of the epidermis rather than between the epidermis and corium. These findings have been confirmed by Cordero (5), Lever (7), Rook and Whimster (9), Tzanck (10–14), and others.

Tzanck not only corroborated this work but proposed a simple procedure which supplements the examination of histologic sections (10–14). By scraping the floor of a bulla one can obtain cellular material for cytologic examination. In these Giemsa-stained smears he demonstrated unique microscopic features which make it possible to distinguish typical acantholytic cells of pemphigus from other epithelial cells. Rook and Whimster have confirmed this work and emphasized the value of cytologic studies in differentiating pemphigus from dermatitis herpetiformis with certainty (9).

The present paper is part of a larger study of the cytology of skin epithelial cells in various disease states. The reliability and value of the Tzanck procedure for the diagnosis of vesicular virus diseases of the skin has been reported earlier from this laboratory (see Fig. 8) (1, 2). Although we do not feel that pemphigus is a virus disease, we are reporting our confirmation of the value of cytologic examination in the diagnosis of the disease as reported by Tzanck, Rook, Whimster, and others.

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TECHNIC

To obtain adequate representative material for microscopic examination the specimen must be taken with great care. Vesicle fluid and blood must be avoided, for they dilute and obscure the epithelial or inflammatory cells which characterize the lesion. The vesicle or bulla chosen for cytologic study should be the youngest one can find, as pus and leukocytes obliterate and lyse the cells of greatest interest. Figure 1 indicates the early lesion desired.

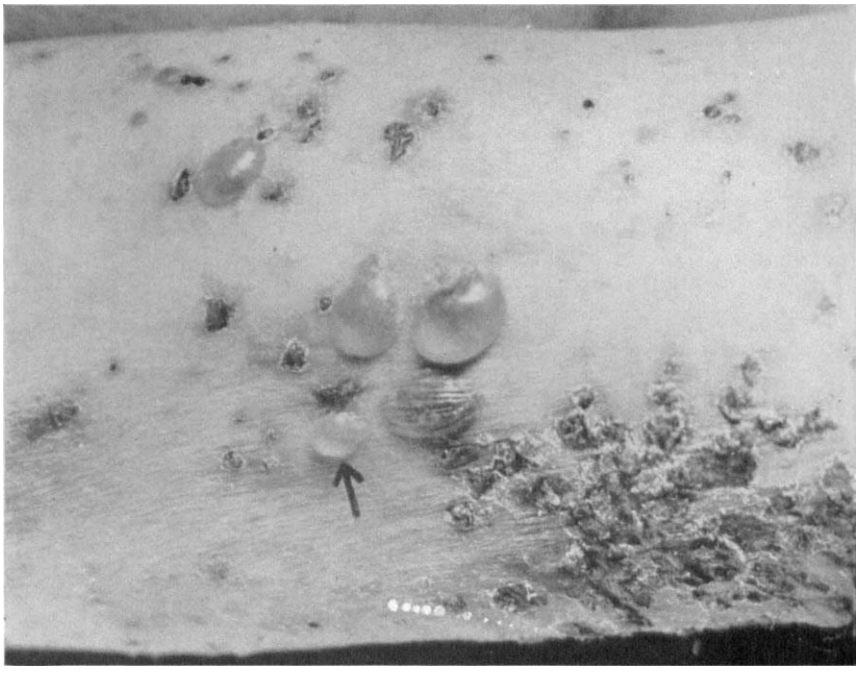

FIG. 1 Pemphigus bullae in various stages of evolution. The early lesion indicated is the type best suited for cytologic study.

The vesicle is gently swabbed with an alcohol sponge and the alcohol allowed to dry. With a scalpel the roof of the vesicle is split open, and with a dry sponge the fluid contents are carefully sponged away without touching its base. The walls of the vesicle are reflected and the base of the lesion is scraped with the sharp edge of the scalpel to remove cellular material. This should be done in one or two strokes before bleeding occurs. The small amount of whitish material is immediately spread without excessive "scrubbing" as a thin layer on a clean glass slide. As soon as the smear has dried in air it is suitable for storage or transport to a laboratory. It will keep for long periods, much as a dried blood film.

The slide is fixed in methyl alcohol and stained with a routine Giemsa stain with the same technic and timing employed for blood smears. We have used a commercial stain which is made up fresh for each batch of slides by adding one

drop of the stock solution to each cc. of neutral distilled water. If the distilled water has become acid the blue colors in the stained slides may seem faded.

After the stained slide has dried in air it may be prepared as a permanent mount by dipping in xylol and applying a cover slip over a drop of balsam. The smear also may be examined after drying, by applying a drop of immersion oil. Oil or balsam should always be applied before even low power microscopic examination, to clear the cells.

All of the cellular material on the slide should be examined under low power to note the abundance of epithelial or inflammatory cells and their relationship to each other.

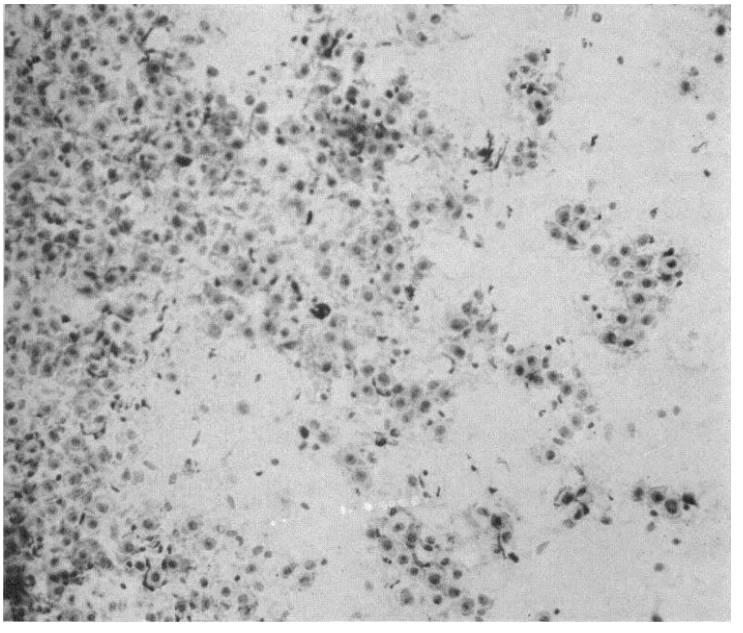

FIG. 2 Smear of base of pemphigus bulla from skin. There is a profusion of epithelial cells, many in loosely adherent clumps. Giemsa ×100.

Microscopic Findings

Smears from the base of pemphigus vulgaris bullae reveal several cytologic features which can be demonstrated more readily than in ordinary histologic sections. *No one abnormality is absolutely specific and all of the features should be observed.*

The most striking difference from all other smears of vesicular or bullous eruptions is the great profusion of epithelial cells in the entire spread when viewed under low power (Fig. 2 and 4). Not only are there more epithelial cells but also the clumps of cells observed differ from the occasionally encountered densely packed clumps of other diseases (Fig. 3, 4 and 5). The cells do not appear to be firmly attached to each other and many of them are isolated or

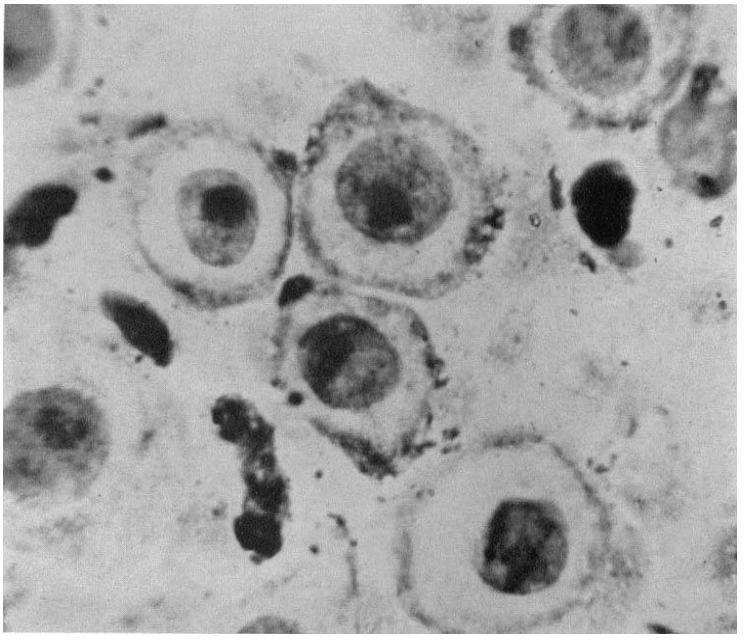

FIG. 3 Smear of base of pemphigus bulla from skin. Acantholytic epithelial cells with a rounded shape, peripheral condensation of cytoplasm, and small amount of cytoplasm in proportion to the nucleus. Giemsa ×900.

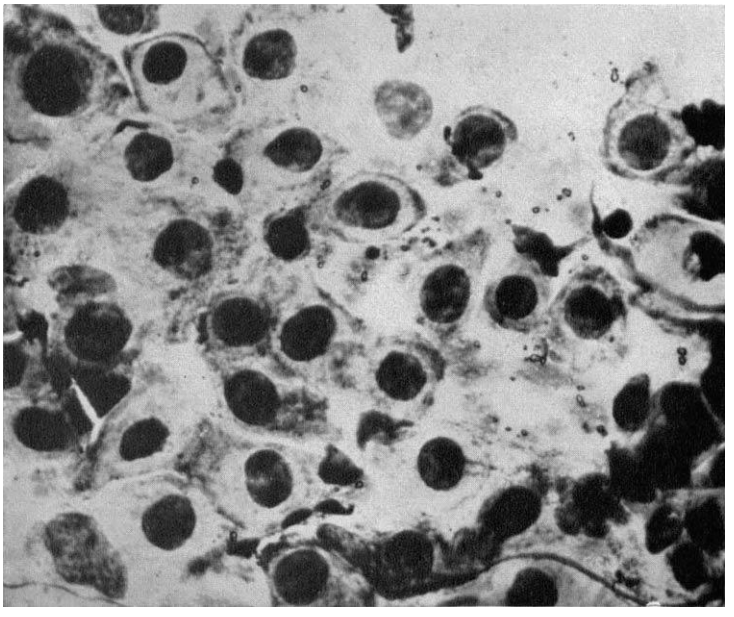

FIG. 4 Smear of base of pemphigus bulla from mucous membrane. The changes are the same as in material from the skin (see Figs. 2 and 3). Giemsa ×600.

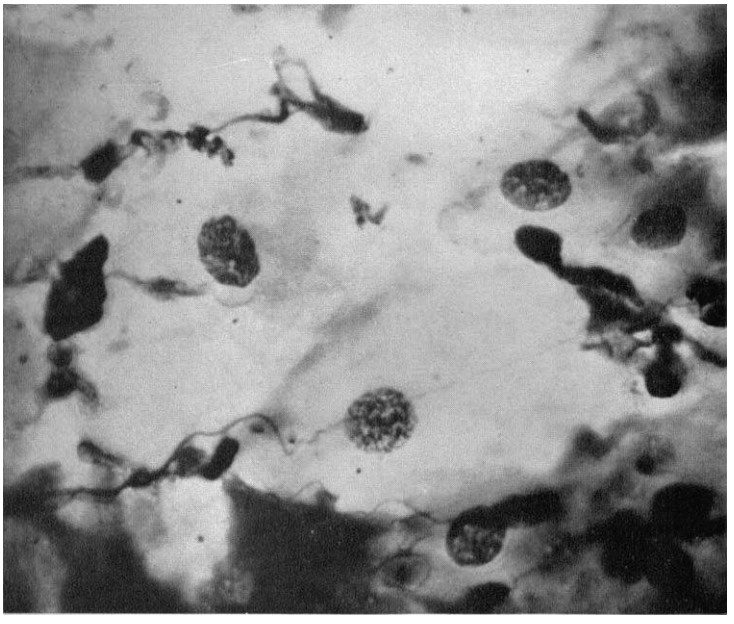

FIG. 5 Smear of normal mucous membrane epithelial cells. Compare with the acantholytic cells of Fig. 4. Giemsa ×600.

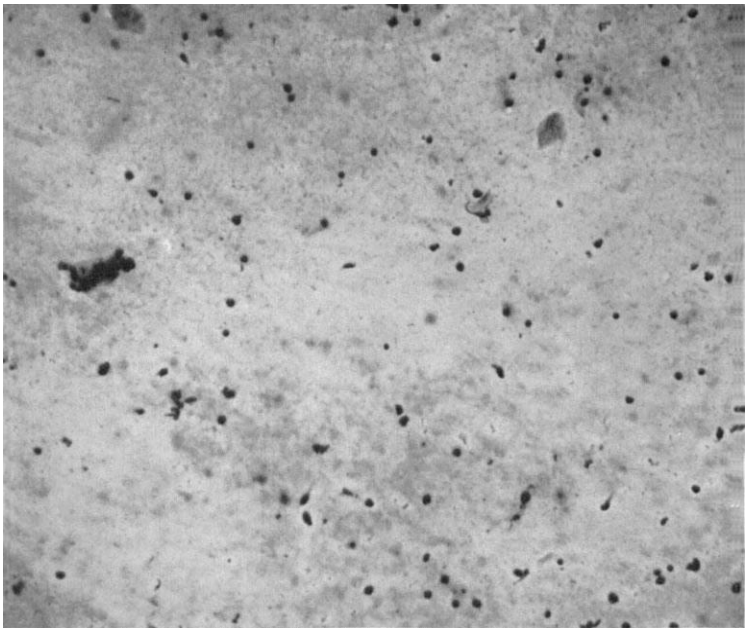

FIG. 6 Smear of base of dermatitis herpetiformis bulla. Inflammatory cells predominate and all cells are sparse. Giemsa ×100.

barely touching their neighboring cells with clear spaces between cells. This is thought to be a manifestation of acantholysis or a loss of prickles and normal cohesiveness.

In addition most of the epithelial cells are a "young" form as ordinarily found in the basal or lower prickle cell layer. They are distinguished by a large nucleus with a relatively small amount of cytoplasm. As a result, the cells seem smaller and have a much larger nucleus to cytoplasm ratio than occurs in the commonly observed broad epithelial cell with small nucleus. One also sees condensation at the periphery of the cytoplasm of the pemphigus epithelial cells which is observed as a darker blue area at the periphery (Fig. 3 and 4).

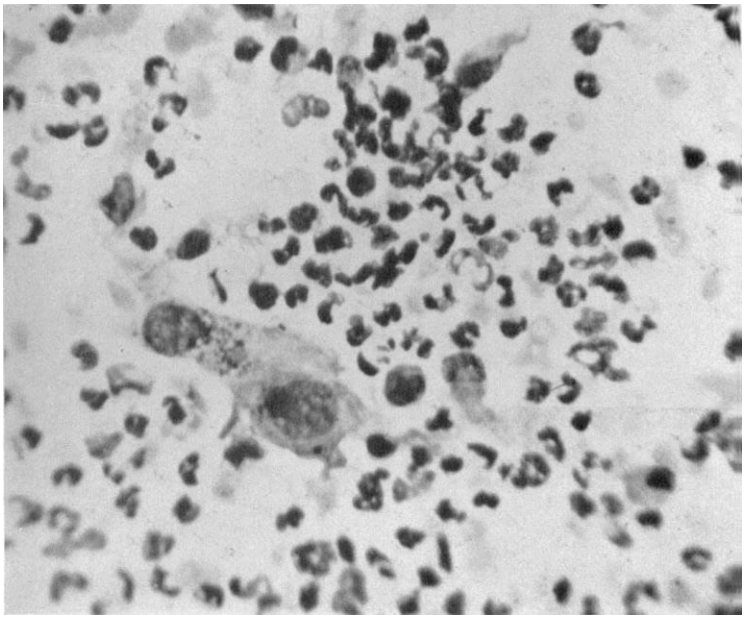

FIG. 7 Smear of base of bulla of drug eruption. Inflammatory cells predominate. The epithelial cells are normal, one of which contains melanin granules. Giemsa ×450.

It is emphasized that an occasional epithelial cell in other bullous diseases will be of the "young" form with a large nucleus, scant cytoplasm, and perhaps peripheral condensation of the cytoplasm but they will be a very small proportion of the total number of epithelial cells present; in contrast to pemphigus where they are very common.

RESULTS

As a part of the general study of epithelial cell cytology a large number of smears as well as histologic sections have been examined from a wide variety of skin diseases including: dermatitis herpetiformis (Fig. 6), erythema multiforme, bullous drug eruptions (Fig. 7), contact dermatitis, bullous impetigo, epidermolysis bullosa, bullous urticaria pigmentosa, thermal burns, cantharides

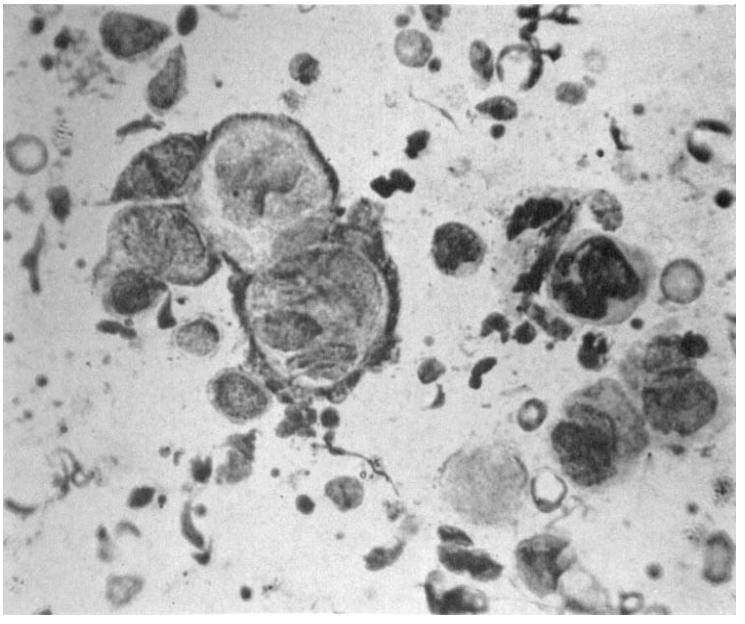

FIG. 8 Smear of base of herpes simplex vesicle. Typical multinucleate giant epithelial cells are numerous. These are readily distinguished from all other epithelial cell changes. Giemsa ×475.

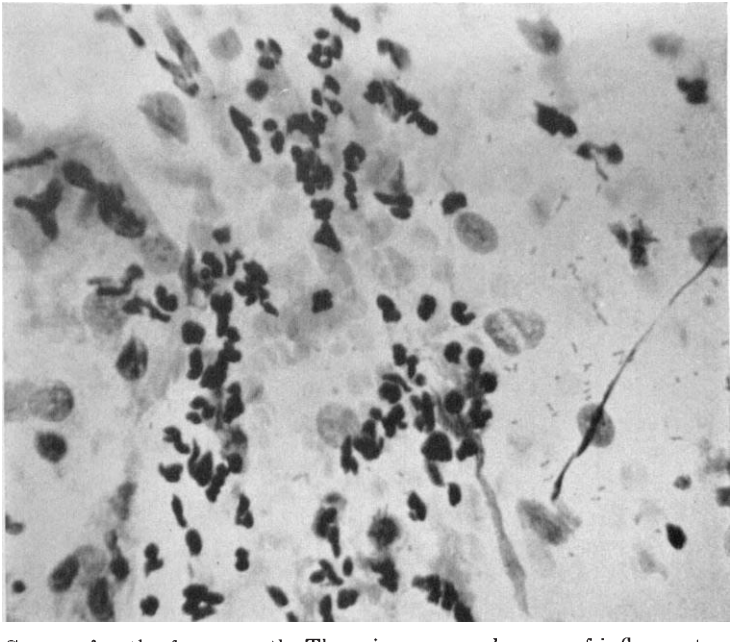

FIG. 9 Smear of aptha from mouth. There is a preponderance of inflammatory cells and the epithelial cells are normal. Numerous bacteria are present. Giemsa ×450.

blisters, dermatophytosis, scabies, recurrent aphthae (Fig. 9), eczematous dermatitis, herpes simplex (Fig. 8), acute herpetic gingivostomatitis, herpes zoster, varicella, vaccinia, et cetera as well as pemphigus vulgaris and chronic benign familial pemphigus (bullous Darier's Disease).

Material from twelve patients with pemphigus vulgaris proved by unequivocal clinical signs and/or subsequent course was studied. The constellation of cytologic features previously described for pemphigus was never encountered in any other disease except bullous Darier's Disease. In this disease the changes are similar but less marked than in pemphigus. The slides from patients with pemphigus were not always diagnostic. Further study revealed that this usually resulted either from failure to choose an early bulla or from faulty technic in obtaining the smear. The most common errors were the smearing of bulla fluid instead of cells and the inclusion of blood. With care in choosing the proper lesion and in performing the technic a diagnostic smear was regularly obtained. As indicated, no false positives were obtained and in skilled hands a negative slide seems highly significant.

DISCUSSION

In the course of our observations we concluded that two previously described observations: acantholysis and intraepidermal position of the bullae, accounted for most of the changes seen under the microscope. The process of acantholysis results in many of the abnormalities in the appearance and shape of the individual epithelial cell. The demonstration of large numbers of epithelial cells at the base of early pemphigus bullae is in contrast with the few epithelial cells obtained by scraping the base of other bullous diseases. One can only conclude from this that in most diseases the bullae are predominantly subepidermal but in pemphigus the bullae are predominantly in the epidermis. Although one can demonstrate in serial sections that pemphigus bullae communicate with the corium it is apparent that there are far more epithelial cells in the floor of these lesions than at the base of other bullae. From our cytologic observations we would agree with the histologic observations that most of the early pemphigus bulla is intraepidermal.

SUMMARY

1. Certain unique microscopic changes are now being recognized in the bullae of pemphigus vulgaris.

2. Tzanck has devised a simple smear technic for readily demonstrating some of the findings originally described in histologic sections by Civatte.

3. In our study of 12 patients with typical pemphigus vulgaris, properly obtained smears taken from early bullae, regularly demonstrated the changes we now regard as diagnostic of the disease. No "false positives" were seen in a large number of smears prepared from a wide variety of other vesicular and bullous diseases.

4. Bullous Darier's Disease (chronic benign familial pemphigus, Hailey, Hailey Disease) shows changes similar to pemphigus vulgaris but to a lesser degree.

5. The characteristic changes in the epithelial cells seen in smears from early pemphigus bullae are:

a. There is a profusion of epithelial cells and relatively few inflammatory cells.
b. Many of the cells seem to be detached or loosely attached to neighboring cells rather than in a tightly adherent sheet (acantholysis).
c. The epithelial cells seem small and have a rounded shape.
d. The nucleus is large in relation to the cytoplasm. (Many of the nuclei are well preserved, with easily distinguished nucleoli.)
e. The cytoplasm usually has a condensed basophilic zone at its periphery.
f. These changes are found in the majority of the epithelial cells and predominate throughout the smear.

6. The demonstration of masses of epithelial cells at the base of early pemphigus bullae, in contrast with the few epithelial cells found at the base of other vesicular and bullous diseases, indicates that most of the early bulla is intraepidermal in pemphigus and subepidermal in other diseases.

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DISCUSSION

Dr. Walter F. Lever, *Boston:* The cytologic test for pemphigus vulgaris is beyond any doubt very important and in skilled hands probably sufficient to establish the proper diagnosis. Nevertheless, I would suggest that whenever possible the cytologic test be supplemented by a true histologic examination.

I do not think that one will ever have difficulties in persuading a patient who potentially has a serious disease like pemphigus vulgaris to submit to a biopsy.

The histologic section shows changes which are even more striking than those of the cytologic test. It is true that the nuclear changes are not as clearly visible as in the cytologic test; but the whole structure of the bulla becomes apparent. The bulla in true pemphigus vulgaris ("pemphigus vulgaris acutus") is intraepidermal and largely located right above the basal cell layer. The primary change is a loss of the intracellular bridges between epidermal cells, a process which is referred to as acantholysis. This acantholysis leads to the formation of intraepidermal slits which gradually enlarge into intraepidermal bullae.

In bullous dermatitis herpetiformis and bullous erythema multiforme the bullae are, as a rule, at least in part subepidermal. In areas in which the bullae are intraepidermal they do not tend to be located right above the basal layer. But most important from the point of view of diagnosis, one never observes acantholysis in these diseases as one does in true pemphigus vulgaris.

DR. BEERMAN, *Philadelphia:* I would like to agree with everything that Dr. Blank has said, and also to agree with everything Dr. Lever has said but would like to go one step further and say, that not only are the cytologic smear and the histologic picture of importance in diagnosis, but it is necessary to have a careful clinical history and examination of the patient along with these other two devices.

In a discussion I had in a paper by Winer and Lipschultz at the American Dermatological Association recently, I think I demonstrated sufficiently well that the same histologic picture may occur in a variety of processes, and it is conceivable that the smears might likewise be similar, but with a careful examination of the patient observing his clinical course and lesions, plus the histologic appearance, plus the smear, we have now I believe for the first time a complete triad of methods by which one may really diagnose true pemphigus.

DR. GANS, *Frankfort:*—I wish to emphasize what my friend, Dr. Beerman just said.

I feel it is very important not to rely too much on clinical, bacteriological, pathological or histological findings. What we have to do, and what is particularly important in such a serious disease as pemphigus is to try to view with the histologically trained eye the clinical aspects of the diseased skin, and to see with a clinically trained eye what histologically we can expect. Otherwise, if we do not have the clinical picture before us, and if we are going to rely only on the histological examination of the smear, we might run the risk to lose credit for what we really know and what we can say in histopathology of the skin. Therefore, I am in full agreement with Dr. Beerman. Have the clinical picture, the histological smears and the histological biopsy to make a diagnosis in this disease which, as you all know is fatal.

DR. FRED D. WEIDMAN, *Phila.:* The success of this technic depends upon the fact that one is examining cells which have not been subjected to the chemical

and physical mauling which they receive in passing through histologic embedding reagents. Its usefulness might be augmented if this work were repeated, but substituting frozen sections for smears; that is, mounting and fixing the frozen section on the slide as though it were a blood film, and Giemsa stain as usual. Thereby one would obviate the distortion and shrinkage of cells in paraffin sections which has heretofore interfered with the recognition of the delicate degenerative changes of the cells in question and which are the crux of the Tzanck technic. Thereby one would have the advantage both of the general architecture and of the patterning which can be secured only in sections, as well as the finer cytologic changes demonstrated by the Tzanck technic.

Dr. Blank very properly emphasized the matter of securing an early lesion, and if the clinician is to expect the fullest help from the pathologist, he must do his part. He must see to it that it is the early lesion that is selected; that is, before secondary degenerative changes due to inflammation or otherwise have entered to complicate the situation. That being the case, I would surmise that any conclusions in respect to the Tzanck tests upon pemphigus cells should be drawn only when similarly early vesicles of dermatitis herpetiformis, contact dermatitis, etc. had been selected. It boils down to this, namely, that the Tzanck test is a study of detailed, fine, degenerative changes of cells, and the cells must be therefore secured in their early unmodified state.

I was intrigued by what Dr. Lever said about spongiosis being observable in very early lesions, i.e. intraepidermal lesions of dermatitis herpetiformis. That comes to me as a surprise. If it could be borne out in frozen sections it would go far toward confirming Dr. Blank's thesis.

DR. BURGOON, *Philadelphia:* I would like to thank Drs. Lever, Gans, Beerman and Weidman for their very kind discussion.

I should like to re-emphasize the point which Dr. Weidman made, namely, the necessity of obtaining a fresh vesicle for the examination. It has been our experience that when negative results are obtained, if we think the patient does have pemphigus, that most frequently this is due to obtaining an old lesion. Usually in this material from old lesions, or lesions that looked new but actually were old, the first thing we find is an absence of epithelial cells. One observes macrophages, polymorphonuclear leukocytes and perhaps some eosinophiles. This brings up an interesting point. One which we think is settled but which has been unsettled for quite a while, namely, the sight of origin of the vesicles or bullae in pemphigus vulgaris. Dr. Lever feels, and I am glad to hear it said again, that these changes start within the epiderm. However there are numerous papers in which the authors state that the bullae start at the junction of the epidermis and dermis. It is obvious, I think, that they had obtained old blisters, and that the changes had already spread down to the epidermal-dermal junction and thereby gave a false impression as to its site of origin. We feel that this technic has perhaps settled that point. It further demells strates that when we take old blisters, we too do not obtain the epithelial conbecause the bulla is resting on the epidermal-dermal junction. This serves to emphasize the point of the importance of obtaining early lesions.